Preantral Follicles of Over 12-Month-Old Mice Have Developmental Potential for Matured Egg Production

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Mammalian ovaries contain numerous numbers of preantral follicles, which decrease with aging throughout lifespan. To utilize these follicles for efficient egg production, the previous studies succeeded in embryo production by in vitro culture of preantral follicles derived from juvenile mice, while there are few reports with successful embryo production in case of aged mice, especially at over 12-month-old. This study aimed to investigate the developmental ability of preantral follicles in aged mice by using our successful protocol in juvenile mice. Preantral follicles between 80 and 120 μm in diameter were obtained from the ovaries of juvenile (0.5-month-old) as a control, and adult (2, 7, 12, and 15-month-old, respectively) mice of BDF1(C57BL/6 × DBA/2). Based on the previous report (Kohama et al., JRD, 2022), the collected specimens were cultured by two steps; as a 1st step, isolated follicles were placed on low cell adhesion membrane inserts under 20% O_2 for 6 days followed by 0.1% collagenase treatment to remove theca cells and basement membranes, and then as a 2nd step culture, formed granulosa cells-oocyte complexes (GOCs) were cultured on high adhesion membrane inserts under 5% O_2 for 6 days. As a result, the survival rates of the preantral follicles in the adult groups were lower than that in the control: 63.2, 54.3, 63.9, and 66.7% in 2, 7, 12, and 15-month-old, respectively, vs. 76.8% in the juvenile. In vitro maturation of cumulus cells-oocyte complexes (COCs) obtained after culture produced matured eggs with lower rates in the adult groups (40.5, 51.0, 34.8, and 48.3% in 2, 7, 12, and 15-month-old, respectively), compared to that in the control (75.2%). Finally, by in vitro fertilization and embryo culture, we found that these matured eggs have potential to produce blastocysts (the blastocyst rates from 2-cell embryos in each group were 75.0, 64.7, 58.3, 100, and 15.4% in 0.5, 2, 7, 12, and 15-month-old, respectively). As far as we know, the present study shows the first evidence that the preantral follicles derived from the ovaries in aged mice over 12-month-old have developmental ability for egg and embryo production.